REMARKS/ARGUMENTS

First, Applicants would like to thank Examiners Long Le and Jacob Cheu for the courtesies extended during the telephonic interview conducted on October 12, 2005.

In response to the Final Office Action of September 12, 2005, Applicants requests re-examination and reconsideration of the instant application. Applicants note that in the aforementioned Final Office Action, the Examiner cites a new reference (Zhang et al. Neurobiology of Aging, Vol. 26, page 207 (2005)) not necessitated by Applicant's amendment (filed August 18, 2005) to support and expand previous arguments. Applicants believe the finality of the action is premature and should be vacated (see MPEP 2164.04).

Applicants respectfully request that this response be entered as some of the claims have been amended herein, thereby reducing the issues for appeal.

Claim Status/Support for Amendments

Claims 1, 39-46 are currently pending. Claims 39, 42, 44-46 have been amended herein. Claims 2-38 were cancelled in a previous response.

As discussed during the aforementioned telephonic interview of October 12, 2005, examined claim 1 (Group I) has been deemed to be allowable by the Examiner. Claims 39-46 are drawn to the non-

elected invention. Applicants respectfully request rejoinder of the remaining claims (39-46), in accordance with the decision in *In re Ochiai*, since the remaining claims (39-46) are limited to the use of the biopolymer markers of claim 1 (the examined claim of the elected Group I invention). If the biopolymer marker peptide of claim 1 is found to be novel, methods and kits limited to its use should also be found novel.

No new matter has been added by the amendments to the claims made herein.

Claim 39 has been amended in response to suggestions made by the Examiner during the telephonic interview of October 12, 2005. For the sake of clarity "in a manner effective to maximize analysis of" in claim 39, step (b) has been replaced with --to elucidate--. Support for this amendment can be found throughout the specification as originally filed, see, for example page 35, lines 19-22.

Claim 42 has been amended to define the acronyms for the recited mass spectrometry procedures. These acronyms are well known to those of skill in the art and are defined in various parts of the specification as originally filed, see, for example page 10, lines 2-11.

Claim 44 has been amended to correspond with the bipolymer maker of claim 1. Support for various types of kits can be found

in the original disclosure, see for example, page 36, lines 9-12; page 47, line 11 to page 48, line 5. Claims 45 and 46 have been amended to provide proper antecedent basis for the term "kit" in claim 44 (as amended herein).

Rejection under 35 USC 112, first paragraph

Claim 1, as presented on August 18, 2005, remains rejected under 35 USC 112, first paragraph, as allegedly failing to comply with the enablement requirement. The Examiner asserts that the claim contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Examiner maintains the assertion that the instant invention would not enable one of ordinary skill in the art to use this invention without undue experimentation. The Examiner reiterates that there is no explanation or illustration of the significance or relationship between the peptide fragments (SEQ ID NO:2) and insulin resistance as seen from the view of the mass spectral profile of the peptides as in Figures 2 and 3. Figure 2 is merely a trypsin digested spectra graph depicting ion 1301, whereas Figure 3 is a trypsin digested spectrum graph depicting ion 1188. There is allegedly no indication which graph represents

insulin resistance patients. There is no indication where are the SEQ ID NOS fragments or the corresponding relationship to insulin resistance. Thus, the Examiner concludes there lacks a scientific nexus between the mass spectrum of the recited SEQ ID NO:2 and the target disease.

Applicants respectfully disagree with the Examiner's assertions.

The "test of enablement" is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the prior art without undue experimentation (see MPEP 2164.01).

As set forth by Applicants in the previous response (filed August 18, 2005), the disclosed method improves the efficiency of mass spectrometry by employing a combination of preparatory steps, e.g. chromatography and 1-D tricine polyacrylamide gel electrophoresis, to increase the amount of biopolymers that can be identified (see the instant specification as originally filed at page 25, line 16 to line 22). In the disclosed method, proteins, (as separated on a tricine gel shown in Figure 1), that are identified as differentially expressed between a disease and a non-disease state are selected for excision (from the gel) and identification (see, for example, page 38, lines 7-11 of the instant specification as originally filed). The protein content of

samples obtained from patients having a history of insulin resistance, or Type I diabetes, is compared with the protein content of samples obtained from patients determined to be normal with regard to insulin resistance and diabetes. Identification of the differentially expressed peptides is accomplished by comparing the peptide mass and fragmentation pattern (mass spectral profile, for example, Figure 3) of the peptides with the peptide mass and fragmentation patterns of known peptides (page 46, line 4- page 47, line 10).

Specifically, the gel photographed in Figure 1 shows a comparison of the protein content of samples obtained from patients having a history of insulin resistance with the protein content of patients determined to be normal with regard to insulin resistance. Band #7 corresponds to a fragment of apolipoprotein A-I precursor protein (as disclosed on right side of original Figure 1.) The identified apolipoprotein A-I precursor protein fragment weighing about 1188.58 daltons corresponds to the biopolymer marker currently claimed; the marker is identified as SEQ ID NO:2 at page 46, lines 4-15. Band #7 appears in all of the insulin resistance (IR) patients, that is, lanes 5 thru 8 (as seen from the left), but not in normal control patients, lanes 1 thru 4. Band #7 was resolved from the gel that is pictured in Figure 1. In other words, Band #7 was identified as "differentially expressed" between

a disease state (insulin resistance) and a non-disease state (normal).

Subsequently, this Band #7 was excised from the gel and subjected to mass spectrometry (TOF MS/MS; Figure 3). The resulting mass spectral profile (sequence) was compared to a database containing known peptides sequences and was identified as a fragment of apolipoprotein A-I precursor protein. The mass spectral profile of SEQ ID NO:2 as shown in Figure 3, as established by the instant invention, can be used as a reference for comparison with test samples. Accordingly, the presence of the mass spectral profile of SEQ ID NO:2 in a sample can potentially identify insulin resistance in the patient from which the sample was obtained, i.e., Figure 3 represents insulin resistance patients.

Thus, contrary to the Examiner's assertions, the instant specification does explain and illustrate the relationship between the claimed peptide fragments in Figure 1 and insulin resistance.

Apparently, the Examiner believes that differential expression is an insignificant characteristic to consider when evaluating a peptide as a potential marker.

A peptide that is identified as differentially expressed between a "disease" state and a "normal" physiological state is more often than not recognized as potential diagnostic marker, even if the involvement of the peptide in the pathology of the disease

is unknown. One of skill in the art would be familiar with this practice since it has been known in the art since at least 1992. See attached abstract of Gunnersen et al., (Proc. of the Natl. Acad. of Sci. USA 89 (24): 11949-53 1992; reference 1) in which the detection of the protein enzyme glutamine synthetase in the cerebrospinal fluid of Alzheimer's disease patients lead to the suggestion of glutamine synthease as a potential diagnostic biochemical marker. Moreover, the Scott D. Patterson article (Physiological Genomics 2:59-65 2000; reference 2 in Response filed June 3, 2005) further demonstrates that it is common practice to select potential disease markers by their differential expression between a disease and non-disease state.

Thus, Applicants respectfully submit that one of skill in the art would find it acceptable to refer to the claimed peptide (SEQ ID NO:2) as a marker based upon its differential expression as seen in Figure 1.

The Examiner appears to point to a recent article (Zhang et al., Neurobiology of Aging, Vol. 26, page 207 (2005)) as evidence of lack of enablement in the instant specification. The Examiner appears to have drawn a direct parallel between the diagnostic method reported by Zhang et al., and the methods described in the instant invention, i.e., proteomic approaches for two-dimensional gel differential electrophoresis coupling with mass spectrometry

analysis. The study by Zhang et al., was aiming to identify biomarkers of common age-related neurodegenerative disease. The authors identified around 30 proteins with >20% change in concentration between older and younger individuals. According to the Examiner, Zhang et al., do not conclude that these proteins as biomarkers, rather, Zhang et al., suggest the data of those proteins are a "value platform" and invite further experimentation and confirmation (see page 214, right column, second paragraph; left column, last paragraph).

In the Response to Applicant's Applicant [sic] section in the outstanding Office action, the Examiner further asserts with respect to Zhang et al., that the discrepancy of protein expression of disease versus normal patients cannot be conclusively confirmed as a biomarker for said disease because Zhang et al., raise concerns on the relationship of the disease and the expression of the peptide fragment- "...is this a cause or consequence of disruption of the blood-brain barrier during aging process as indicated by others?" (See page 214, left column, second paragraph). In addition, Zhang et al., point to a disadvantage of this approach as the researchers were unable to discern if the differences found were due to age-related changes that were highly abundant in a single individual, focused in only a few individuals, or distributed over all participants. The Examiner states that

development of mass spectrometry analysis has progressed for decades, yet in the recent article of Zhang et al., uncertainty still remains and further studies are need to confirm the validity of this approach (see page 214, right column, last paragraph). Thus, the Examiner concludes in view of the aforementioned lack of predictability in the art, undue experimentation would be required to practice the claimed methods with reasonable expectation of success.

Applicants respectfully disagree with the Examiner's reliance on the article by Zhang et al.

As noted by the Examiner, Zhang et al., was published in 2005 (received October 2003), which is more than 3 years after the filing date of the instant invention (November 23, 2001), thus, Applicants believe the reference is not relevant to the state of the art existing at the filing date of the application and cannot be used to determine whether the instant disclosure is enabled as of the filing date. It has been established that, in general, the Examiner *should not* use post-filing date references to demonstrate the patent is non-enabling (see MPEP 2164.05(a)).

Assuming, *in arguendo*, Zhang et al., is a valid reference the mere fact that something has not been done clearly is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it (see MPEP 2164.02).

Applicants assert that upon closer inspection the study of Zhang et al., do not parallel the methods disclosed in the instant invention. Zhang et al., used a "shotgun" proteomic approach coupled with liquid chromatography followed by mass spectrometry to identify proteins in human CSF (see abstract; page 208, left column, last paragraph). Specifically, the method of Zhang et al. used pooled CSF samples from 22 younger and 16 older subjects to generate two pooled samples for proteomic analysis (page 208, right column, penultimate paragraph). Zhang et al., acknowledges the problem with the use of pooled CSF samples is that they were unable to determine if the differences found were due to age-related changes in a single individual, only few individuals or distributed over all participants (page 214, last paragraph).

The claimed methodology of the present invention does not use pooled samples, rather, a sample from an individual patient is obtained and at least one biopolymer marker sequence is isolated from the sample and compared to the biopolymer marker sequence as disclosed in the present invention. Unlike Zhang et al., the presence of the mass spectral profile of SEQ ID NO:2 of the instant invention in a sample can potentially identify insulin resistance in the patient from which the sample was obtained, see for example, page 46, line 22 to page 47, line 10.

The Zhang et al. publication states the aging *markers* (30

identified proteins) need to be validated (page 211, right column 2nd full paragraph). Only two proteins (agrin and hnBNPm) were then identified individually by Western blot analysis. Zhang et al., refers throughout the publication to these proteins (agrin, hnBNPm) as "protein markers", see, for example page 211, right column, 2nd full paragraph, lines 1-6 and last line; page 214, right column, lines 2-4. Thus, contrary to the Examiner assertion, Zhang et al., do refer to these identified proteins as potential markers, but invites further study. Therefore, Zhang et al., actually supports and validates Applicants' study.

Furthermore, as stated in Zhang et al., the researchers used 2D gel electrophoresis/MS-base proteomic analysis in conjunction with isotope-coded affinity tags (ICAT) which bind to cysteinyl groups of all cysteine-containing proteins (at page 208, left column, third full paragraph to right column first paragraph). The Zhang et al., article warns that the ICAT technique is not without limitations, the major one being that the ICAT is limited to cysteinyl-containing proteins, thereby focusing quantification on this subset of proteins only (see page 208, sentence bridging left and right columns.) As can be seen by the Sequence Listing (filed April 19, 2002), none of the SEQ ID NOS:1-5 disclosed in the instant invention contain cysteine residues in the protein fragments.

Thus, Applicants respectfully submit that the Zhang et al., reference is not analogous to the instant invention and should not be used to control enablement.

The guidelines for a "test of enablement" indicated that if a statement of utility in the specification contains within it a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated, 35 USC 112, is satisfied (see MPEP 2164.01(c)). The instant application discloses a method for diagnosing insulin resistance through the detection of the claimed biopolymer marker, SEQ ID NO:2. The data presented in Figures 1, 3 clearly show a positive correlation between the claimed biopolymer marker and insulin resistance. These bipolymer markers have not previously been shown to be associated with insulin resistance. When a marker is discovered to be associated with a disease state, its potential for diagnostics and/or therapeutics is immediately recognized, even if the involvement of the marker in disease pathology is unknown.

As established by the above arguments, the instant specification, contrary to the Examiner's opinion, does contain proper guidance to enable one of ordinary skill in the art to practice the claimed method for diagnosing insulin resistance without undue experimentation. Thus, the Examiner's argument is not sufficient to sufficient to support the enablement rejection;

Thus, Applicants respectfully submit that the Zhang et al., reference is not analogous to the instant invention and should not be used to control enablement.

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As established by the above arguments, the instant specification, contrary to the Examiner's opinion, does contain proper guidance to enable one of ordinary skill in the art to practice the claimed method for diagnosing insulin resistance without undue experimentation. Thus, the Examiner's argument is not sufficient to support the enablement rejection; since the

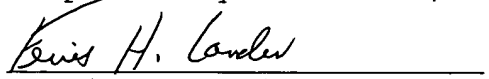
association of the claimed bipolymer marker, SEQ ID NO:2, with insulin resistance carries with it a connotation of use for diagnostics. Moreover, the decision in *In re Brandstadter* (179 USPQ 286) has established that the evidence provided by Applicants (to overcome an enablement rejection) need not be conclusive but merely convincing to one of skill in the art (see MPEP 2164.05).

In conclusion, Applicants respectfully submit that the instant specification, as originally filed, provides a clear explanation of the relationship between the recited peptide (SEQ ID NO:2) and insulin resistance. Thus, Applicants respectfully request that this rejection under 35 USC 112, first paragraph now be withdrawn.

CONCLUSION

In light of the foregoing remarks, amendments to the specification, and amendments to the claims, it is respectfully submitted that the Examiner will now find the claims of the application allowable. Favorable reconsideration of the application is courteously requested.

Respectfully submitted,



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NCBI PubMed National Library of Medicine NLM

Reference #1

1: Proc Natl Acad Sci U S A. 1992 Dec 15;89(24):11949-53.

Related Articles, Links

Detection of glutamine synthetase in the cerebrospinal fluid of Alzheimer diseased patients: a potential diagnostic biochemical marker.

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In this report, 8- and 2-azidoadenosine 5'-[gamma-32P]triphosphate were used to examine cerebrospinal fluid (CSF) samples for the presence of an ATP binding protein unique to individuals with Alzheimer disease (AD). A 42-kDa ATP binding protein was found in the CSF of AD patients that is not observed in CSF from normal patients or other neurological controls. The photolabeling is saturated with 30 microM 2-azidoadenosine 5'-[gamma-32P]triphosphate. Photoinsertion can be totally prevented by the addition of 25 microM ATP. Photoinsertion of 2-azidoadenosine 5'-triphosphate into the protein is only weakly protected by other nucleotides such as ADP and GTP, indicating that this is a specific ATP binding protein. A total of 83 CSF samples were examined in a blind manner. The 42-kDa protein was detected in 38 of 39 AD CSF samples and in only 1 of 44 control samples. This protein was identified as glutamine synthetase [GS; glutamate-ammonia ligase; L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] based on similar nucleotide binding properties, comigration on two-dimensional gels, reaction with a polyclonal anti-GS antibody, and the presence of significant GS enzyme activity in AD CSF. In brain, GS plays a key role in elimination of free ammonia and also converts the neurotransmitter and excitotoxic amino acid glutamate to glutamine, which is not neurotoxic. The involvement of GS, if any, in the onset of AD is unknown. However, the presence of GS in the CSF of terminal AD patients suggests that this enzyme may be a useful diagnostic marker and that further study is warranted to determine any possible role for glutamate metabolism in the pathology of AD.

PMID: 1361232 [PubMed - indexed for MEDLINE]